BIO_08 - Optimizing sample preparation and nLC-MS/MS for mAB characterization

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Introduction: Monoclonal antibodies (mAB) are highly effective drugs for treatment of cancer and other diseases. Production and development of mAB require physicochemical characterization of several Critical Quality Attributes (CQA), including post-translational-modifications, disulfide bond and amino acid primary sequence mapping. In this study we aim to use design of experiments (DoE) to improve both sample preparation and nLC-MS/MS analysis by decreasing protein digestion time, artificial amino acid modifications, increasing protein sequence coverage and analysis throughput. We aim to develop an optimized nano LC method with in- house produced columns capable of characterizing CQA of mAB.

Objectives: Optimization of protein coverage and relative quantification of PTM by nLC-MS/MS.

Methodology: Nivolumab and pembrolizumab were used in this study. For DoE, a full factorial 2^2 or 2^4 with or without central point were used. For protein digestion protocol, different parameters were compared: time of digestion, digestion buffer and denaturation agent. Chromatographic conditions were tested, varying gradient and sample injected volume for both mAB peptide and disulfide bond mapping methods by nLC-MS/MS. The nLC- MS/MS was carried out on an Ultimate 3000 coupled with an Orbitrap Exploris 120. Peptides were sequenced using Biopharma Finder 5.1.

Results: DoE is incredibly challenging for optimization of peptide mapping protocols. Several responses must be considered to compare different analysis conditions. Protein coverage, by itself, is a poor response for method evaluation. The use of guanidine HCl without desalting prior to digestion decreases digestion efficiency but increased light chain coverage. Digestion time of 1h reduces asparagine deamidation by over 30x in comparison to 18 h. $CaCl^2$ as a stabilizer for trypsin didn't increase the efficiency of protein digestion. Sequencing grade trypsin can be used for 1 hour at a temperature of 47 °C. Other temperature conditions may be tested. The use of 50 mM Tris-HCl pH 8.0 as a digestion buffer instead of ammonium bicarbonate had no impact on the results of peptide mapping of nivolumab. The gradient of 120 min for separation of protein digest showed no advantage over 60 min gradient.

Conclusion: A robust peptide mapping by LC-MS/MS method can be achieved with 60 min digestion and 60 min gradients with high protein coverage and little artificial modification of residues due to sample preparation using nLC and in house produced columns.

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